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Award Number: DAMD17-00-1-0030

TITLE: Androgen Ablation Combined with CTLA-4 Blockade-Based Immunotherapy as a Treatment for Prostate Cancer

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REPORT DATE: January 2003

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20030904 043

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 2003	Final (1 Jan 00 - 31 Dec 02)

4. TITLE AND SUBTITLE
Androgen Ablation Combined with CTLA-4 Blockade-Based Immunotherapy as a Treatment for Prostate Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0030

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Manipulations capable of repealing host tolerance to induce T cell-mediated prostate tissue-specific responses are of central importance to immunotherapeutic approaches to prostate cancer treatment. Hence in the current proposal, we test whether androgen ablation (by castration) can induce T cell responses targeting murine prostate epithelial and tumor cells. We previously showed that castration of TRAMP mice results in prostate and tumor infiltration by antigen presenting cells (APC's) as well as CD4+ and CD8+ T cells. These studies completed our original **Specific Aim 1** and suggested that castration can prime host responses amenable to immunotherapeutic potentiation. Over the last year we have essentially addressed **Specific Aims 2 and 3** by showing that androgen ablation can facilitate/potentiate the effectiveness of novel immunotherapies (anti-CD40 and anti-CTLA-4 treatment) to enhance T cell-mediated TRAMP tumor inflammation for prostate cancer treatment. Hence, we have extended our studies by conducting a number of exploratory experiments to elucidate central mechanisms whereby androgen ablation augments host T cell-mediate immunity.

14. SUBJECT TERMS:	15. NUMBER OF PAGES
prostate cancer	9
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Our previous studies pertaining to T cell costimulatory-based immunotherapies of murine prostate cancer [1-9], as well as antitumoral immunotherapeutic studies by our collaborators and others [10-14], underscore two principle points: 1) immunotherapies (including CTLA-4 blockade) may prove particularly effective when tumor-induced impairments in host immune function are minimized -- for instance, by diminishing tumor burden and/or reducing the capability of tumor cells to elaborate immune inhibitors; and 2) prior interventions that successfully overcome host tolerance to prime a prostate-specific, cell-mediated immune response should greatly facilitate antitumoral responses bolstered by immunotherapies such as CTLA-4 blockade. Hence, to address these two points within the context of developing potent immunotherapeutic approaches for the treatment of prostate cancer, we originally proposed to test whether androgen ablation might prove useful as a "first step" in converting prostate tumors into their own "*in situ vaccines*" to facilitate a response to immunotherapy. The mechanism(s) we envisioned whereby androgen ablation might induce/facilitate immunotherapeutic responses are encompassed in two non-mutually exclusive paradigms. In the first paradigm, androgen ablation reduces tumor burden thus reducing the number of tumor cells that must be eliminated by an immune response raised by CTLA-4 blockade. Additionally, reduction of tumor burden or downregulation of tumor cell function by androgen ablation may partially alleviate some immuno-suppressive effects of the tumor upon APC and T cell function. In the second paradigm, androgen ablation results in the recruitment of immune cells into tumor sites. Moreover, androgen ablation-induced prostate cell death might result in greater availability tumor-derived antigens that can be appropriate by host APC for presentation to induce antigen-specific activation of T cells whose action can be potentiated by immunotherapies such as CTLA-4 blockade – somewhat analogous to the capability of CTLA-4 blockade to markedly exacerbate pre-existent pathologic inflammatory processes such as murine experimental allergic encephalomyelitis.

BODY

Our current DOD proposal is intended to address our overall hypothesis that: **Androgen ablation combined with CTLA-4 blockade immunotherapy can diminish/prevent the progression of prostate cancer that inevitably occurs following androgen ablative therapy alone.** The following annual report summarizes our progress between January to December 2001 in testing this overall hypothesis by addressing three separate aims we previously outlined:

Specific Aim 1. To Histologically and functionally characterize immune cells that infiltrate prostate tumors following androgen ablation.

Specific Aim 2. To test the hypothesis that *in vivo* CTLA-4 blockade can diminish the progression of prostate cancer that inevitably occurs following androgen ablative therapy alone.

Specific Aim 3. To test the hypothesis that in vivo activation of host antigen presenting cells can potentiate antitumoral responses raised by the combination of androgen ablation and CTLA-4 blockade immunotherapy.

In year 1 of this project, we completed the objectives of our original *Specific Aim 1.* Data from these experiments showing that castration raises T cell-mediated inflammation within the murine prostate were comprehensively summarized in our previous Technical Report to the DOD for the interval between January and December 2000. Over the last year, we have been conducting experiments, as outlined in our **Statement of Work,** to complete experiments outline in our original *Specific Aims 2 and 3.* These experiments requiredhybridoma production of 22 mg of anti-CTLA-4 (9H10 cells) monoclonal antibody, the breeding homozygote TRAMP mice with C57BL/6 mates to generate 150 heterozygote males for establishment of treatment cohorts, and hybridoma production of 210 mg of AntiCD40 rat monoclonal antibody (10C8 cells). ***In year 2***, we demonstrated that castration-induced T cell-mediated inflammation in the prostates of TRAMP mice is apparently potentiated by systemic (intraperitoneal) combined anti-CD40 and anti-CTLA-4 administration. In these experiments, cohorts of twelve week-old adult post-pubertal TRAMP mice (n=25 each cohort) underwent castration on Day 0 of the experiment. Non-castrated cohorts of mice served as non-androgen ablated controls for this study. At day 3 following castration, the treatment cohorts received a single IP injection of anti-CD40 antibody (500 ug) to promote host APC activation -- including especially those APC's infiltrating prostate tissues following castration. Control (non-immunotherapy-treated) cohorts received irrelevant control IgG. Furthermore, mice that received anti-CD40 additionally received I.P. injections of 100 ug of anti-CTLA-4 on days 4, 7 and 10 following castration. Likewise, non-immunotherapy-treated mice received corresponding control IgG. All mice were sacrificed two weeks following completion of antibody treatment. Immunohistochemical analysis of prostates recovered from non-castrated TRAMP mice revealed that combined anti-CD40 + anti-CTLA-4 treatment failed to significantly increase levels of prostate CD3 T cells within the prostate. In contrast, and as we have previously shown, non-immunotherapy-treated castrated TRAMP mice revealed increased levels of prostate CD3 T cells. Finally, administration of combined anti-CD40 + anti-CTLA-4 treatment to castrated TRAMP mice appears to further enhance levels of CD3 T cells infiltrating the TRAMP prostate. ***In year 3***, we initiated a number of exploratory experiments to extend our studies to elucidate mechanisms whereby castration might induce prostate inflammation to facilitate immunotherapeutic responses within the prostate. These extended studies are of particular interest to us since we have recently shown (and published [15]) that androgen ablative therapy in men with prostate cancer raises T cell-mediated prostate inflammation similar to that observed in the TRAMP model (as well as in normal C57BL6 mice). Our current line of investigation is to test the general hypothesis that androgen withdrawal augments host T cell-mediated immunity by lowering the overall threshold for T cell co-stimulatory activation/proliferation. The impetus for this avenue of study has been provided by previously published reports that: 1) castration of post-pubertal animals can potentiate B and/or T cell-mediated responses against various pathogens or tissue allografts [16]; 2) castration profoundly stimulates T and B cell lymphopoiesis in postpubertal rodents [16, 17] -- implying that certain restrictions on lymphocyte proliferation and/or activation might be lifted upon withdrawal of androgen and; 3) clinical prostate cancer progression might be modulated by systemic host immune responses apparently triggered upon androgen deprivation [18-21]. During the course of these experiments we have found that T cells infiltrating normal and TRAMP murine prostate tissues exhibit clonotypic TCR Vβ 8.3 gene usage (as is observed in men with prostate cancer), consistent with prostate tissue/tumor-specific responses triggered by androgen deprivation. Hence, we have extended our studies to elucidate mechanisms whereby castration might induce prostate T cell inflammation. One hypothesis that we are presently testing is that androgen withdrawal augments host T cell-mediated immunity by lowering the overall threshold for T cell co-stimulatory activation/proliferation. In support of this hypothesis, we have been able to demonstrate that T cell and B cell (IgG) responses against the nominal antigen, TNP-OVA, are enhanced by castration (androgen withdrawal) in vaccinated C57BL/6 mice. Similarly, T cells recovered from castrated mice appear to exhibit increased reactivity to alloantigens (in allo-MLR). Also, anti-TRAMPC1 T cell-mediated responses are enhanced by castration in mice primed (vaccinated) with GM-CSF/B7-expressing TRAMPC1 vaccines. We have further observed that castration produces an increase in host levels of B lymphocytes

and T cells *in vivo* as evidenced by a broad and uniform increase in CD4+ and CD8+ T cell numbers within thymii, lymph nodes and spleens of castrated mice. To further establish a mechanistic basis for the enhanced T cell reactivity that is observed following castration, in recent studies we have observed that T cells recovered from castrated mice proliferate at much higher rates in response to variable concentrations of anti-CD3/anti-CD28-mediated costimulation relative to T cells recovered from sham-castrated mice. Moreover, we have observed that T cells from castrated mice exhibit enhanced responses to accessory costimulation provided by ligation of T cell OX40 and/or 4-1BB receptor. **These observations are summarized in a recently prepared manuscript that is currently under review [*see below* In Preparation Section, ref. A].** Finally, using differential gene expression methods, we have determined that enhanced responses by T cells from castrated mice apparently arise from alterations in expression of T cell signaling and transcriptional genes. At present, we are validating these gene changes by QT-PCR and western blot analysis. Collectively, the above studies suggest that castration lowers the overall threshold that is required for T cell costimulatory activation. Such studies provide compelling support for the potential of androgen withdrawal as a viable means to facilitate/potentiate responses to immunotherapy.

In summary, our studies strongly support a potential for CTLA-4 blockade-based immunotherapy as a treatment for prostate cancer. Moreover, we have now demonstrated that standard therapies for advanced prostate cancer (i.e. androgen withdrawal) may greatly facilitate immunotherapeutic responses. Such improvements in prostate cancer treatment are desperately needed to enhance treatment responses and patient survival-- especially for those patients advanced prostate cancer. Thus, it is hoped that our studies will ultimately prove extremely important to the ultimate goal of improving advanced prostate cancer treatment (by immunotherapy) as well as our overall understanding of the immunobiology of prostate cancer.

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KEY RESEARCH ACCOMPLISHMENTS

- Completed Specific Aim 1, 2 & 3. Established that castration in TRAMP mice induces prostate tumor infiltration by mononuclear inflammatory cells.
- Initiated exploratory studies to examine the effects of androgen withdrawal on T and B cell regulation/function.

REPORTABLE OUTCOMES TO DATE

1. **Kwon ED**, Foster BA, Hurwitz AA, Madias C, Allison JP, Greenberg NM, Burg MB. Elimination of residual metastatic prostate cancer following surgery and adjunctive CTLA-4 blockade immunotherapy. *Proc. Natl. Acad. Sci.*, 96(26):15074-9, 1999.
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In Preparation

A. Roden AJ, Moser MT, Mercader M, Hurwitz AA, McKean D, Celis E, Tindall D, Leibovich B, Weinberg A, Chen L, Allison JP and **Kwon ED**. Castration lowers T cell activation thresholds and increases lymphocyte levels to enhance specific immunity. In review 2003.

B. Mercader M, Bodner, B, Manecke R, Wojcik E, Moser M, Kwon PS, Flanigan RC, Waters WB, and **Kwon ED.** Early changes in human prostate gene expression and histology in response to combination androgen ab

CONCLUSIONS

Observations emanating from our current studies collectively support the hypothesis that castration causes prostate tumor infiltration by both antigen presenting cells and T cells with potential antitumoral effector capabilities. Such a response might serve as a useful "*first step*" to prompt host prostate tumors (both primary or metastatic) to act as their own *in situ* vaccines to "*jump-start*" anti-prostate cancer immune responses. To date, we have characterized this response and have shown that two promising immunotherapies, CTLA-4 blockade and/or anti-CD40 treatment, can act synergistically with castration to induce T cell-mediated responses against prostate tumors in the immunocompetent TRAMP mouse. Our work has further provided the basis for clinical phase I testing of CTLA-4 blockade for the treatment of prostate cancer. Additionally, our current studies have provided the scientific foundation for a DOD-sponsored clinical phase II trial that will begin later this year to test whether androgen ablative therapy + anti-CTLA-4 administration can be used to induce/potentiate T cell-mediated anti-prostate tumor responses in men with advanced prostate cancer. Finally, our current exploratory studies may elucidate additional mechanisms whereby hormone therapy modulates T cell-mediated immunity. Such studies may reveal additional novel strategies to markedly potentiate antitumor responses during immunotherapeutic intervention during prostate cancer treatment.

APPENDICES: NONE